Emerging Technologies in Sample Analysis

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New England Bioterrorism Preparedness Workshop

MIT Lincoln Laboratory

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Abstract

Subject Terms

Report Classification unclassified	**Classification of this page** unclassified
Classification of Abstract unclassified	**Limitation of Abstract** SAR

Number of Pages
19

Outline

- **Current techniques in sample analysis**
  - **Clinical (subject of yesterday's talk)**
  - **Environmental**
- **Challenges associated with environmental sampling**
- **Examples of technologies in use and in development**

CDC's Sample Analysis Guidelines
(example: *B. Anthracis*)

- **Persons suspected of exposure/infection**
  - Cultures of blood and spinal fluid
  - Cultures of tissues or fluids from affected areas
  - Microscopic examination
  - PCR
  - Nasal swab (occasionally for exposure, but not for diagnosis)
  - Antibody testing (exposure, not validated for diagnosis)

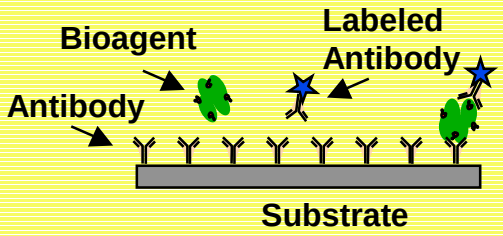
- **Environmental contamination**
  - Cultures of air samples, surface swabs, suspicious powders
  - Microscopic examination of suspect material
  - Evaluation of growth properties of suspect agent
  - PCR
  - DFA (direct fluorescent assay) to detect key bacterial proteins
  - Specialized tests, such as immunoassays (SMART)

How Do These Techniques Compare?

Response Time

Rapid ID

Immunoassays

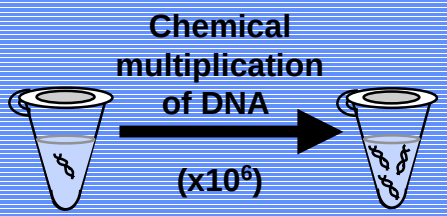


- Selectivity from high affinity binding of antibody to agent-specific structures

~15 minutes

Orthogonal ID Confirmation Technologies

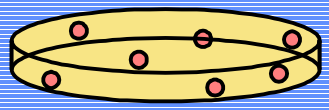
Polymerase Chain Reaction (PCR)



- Selectivity from sequence-specific DNA/RNA recognition
- Enzymatic amplification provides superb sensitivity

1- 4 hrs

Culture-based assays



- Traditional method since Pasteur – still "gold standard" for ID
- Viable organisms replicated in culture and identified using biochemical assays and microscopy

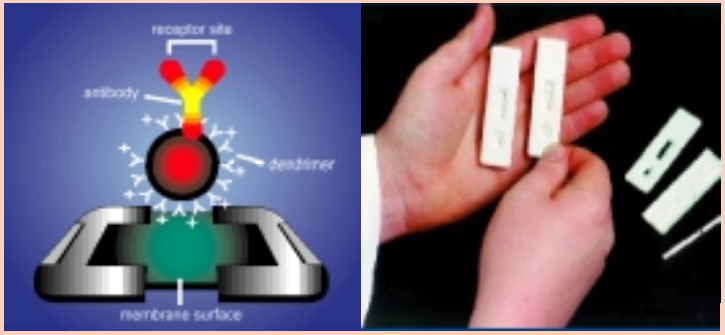
1-3 days

Sensitivity/Accuracy

Examples of In-use and Developmental Immunoassay Devices

Ticket cartridges and reader for lateral-flow immunoassay in Joint Biological Point Detection System (JBPDS)

Response Equipment Co. Bio-HAZ Biodetector



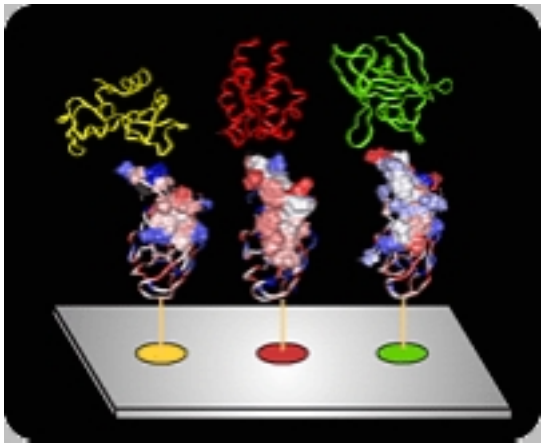
Dendrimer-Based Alert Ticket (ARL)

Upconverting Phosphors (SRI)

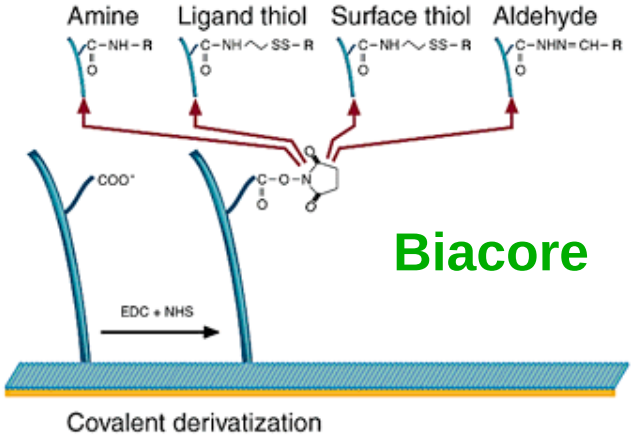
Features of Immunoassay Analysis

- Can be used on environmental samples with little or no preparation
- Readout is fast (~ 15 minutes) and simple (colorimetric or fluorimetric)
- Sensitivity modest (~10,000 - 100,000 particles)
  - Depends on antibody-antigen binding affinity and readout scheme
- Specificity reasonably good
  - Depends on antibody construct and antigen specificity
- Current IAs are not multiplexed; development of protein microarrays may lead to sensitive, multi-assay analysis tools

Examples of Existing Protein Microarrays

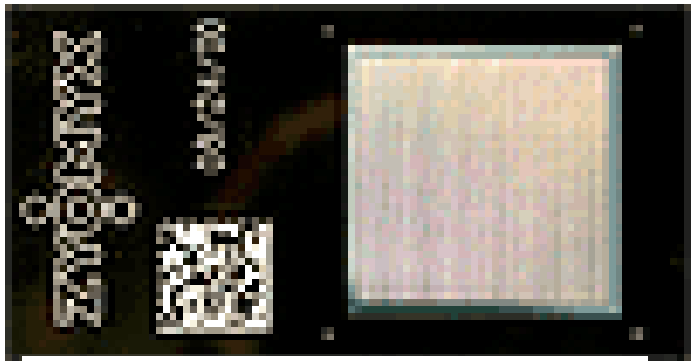


Phylos (2000 element)



Biacore

Ciphergen (multiple classes of proteins)

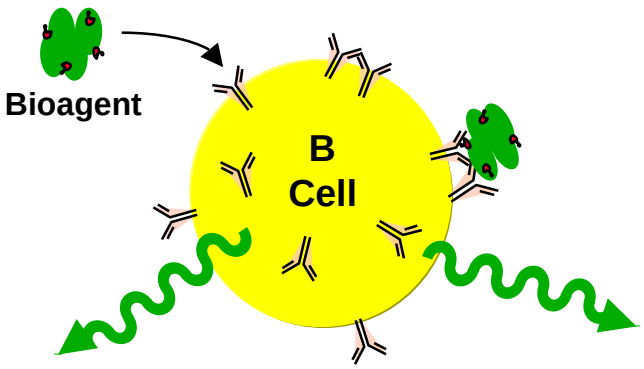


Zyomyx (10,000/cm^2)

- Protein microarray technology development driven by drug screening and disease-marker investigations
  - Diagnostics (clinical and environmental) still developmental

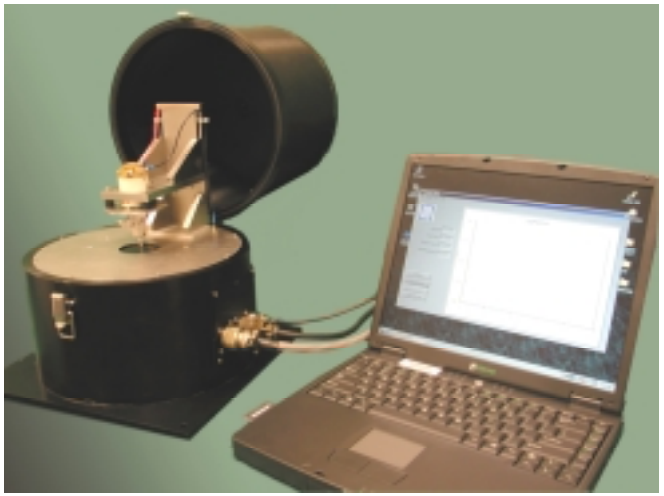
Developmental Antibody-Based Sensor: CANARY

Concept



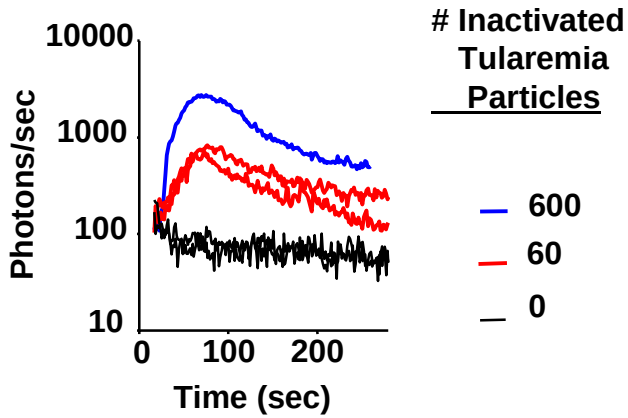
B cell emits ~200 photons within 30 seconds after bioagent binding

Prototype microcentrifuge device



Tests Against Killed Tularemia

(Collab. with NMRC)



Status of B-Cell Lines

Complete	In development
FMDV	*Coxiella burnetti*
VEE	*Bacillus anthracis*
Vibrio cholera	*E. coli* O157:H7
Orthopox viruses	
Yersinia pestis	
Brucella spp	
Francisella tularensis	

PCR-Based Analysis Tools

- **Systems being developed (and deployed) that provide agent ID within 30 minutes of introduction of prepared sample**

Semi-automated field-portable PCR devices

RAPID - Idaho Technologies

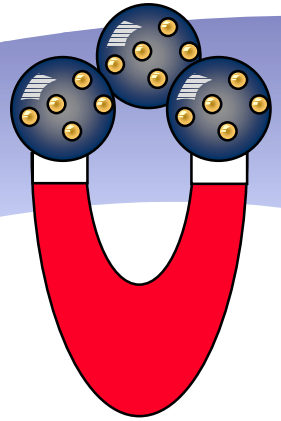
SmartCycler
XC System - Cepheid

Example of handheld PCR device

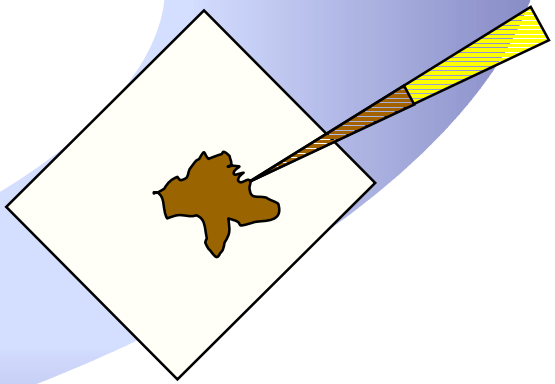
HANAA - Handheld Nucleic Acid Analyzer, developed by LLNL, Cepheid, and ETG, Inc.

- **Challenge remains in automating sample preparation and analysis**
  - **Pathogen cells or spores must be ruptured to liberate the DNA/RNA**
  - **DNA/RNA must be separated from protein debris/environmental impurities**

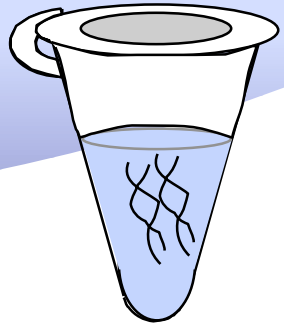
Overview of Sample Preparation



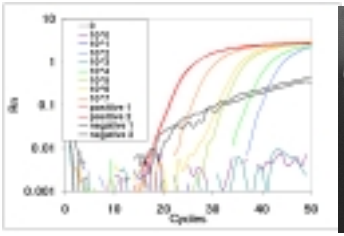
2) Concentration

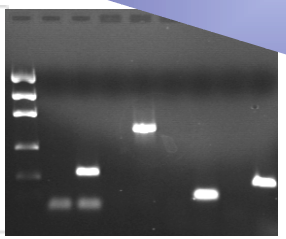


3) Purification



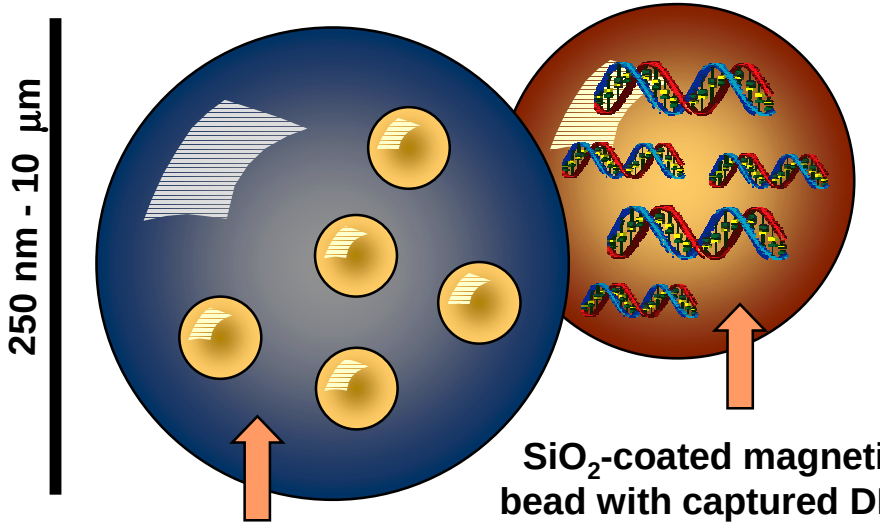
4) Chemical Amplification

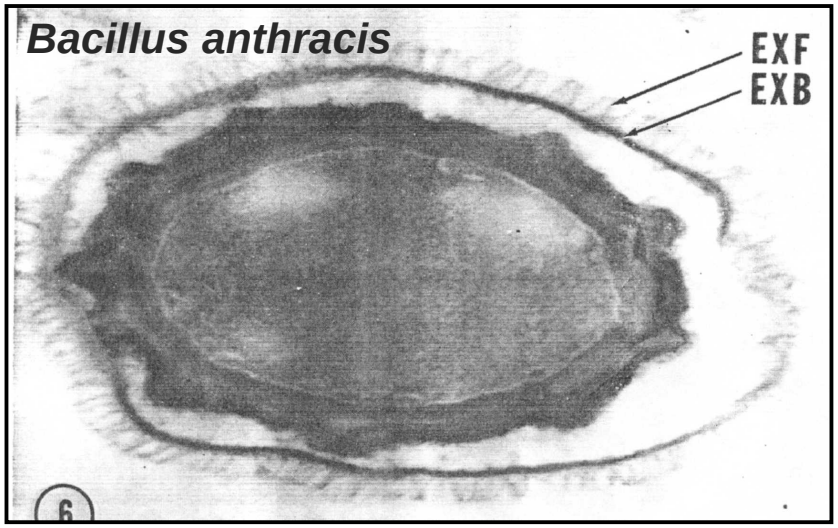


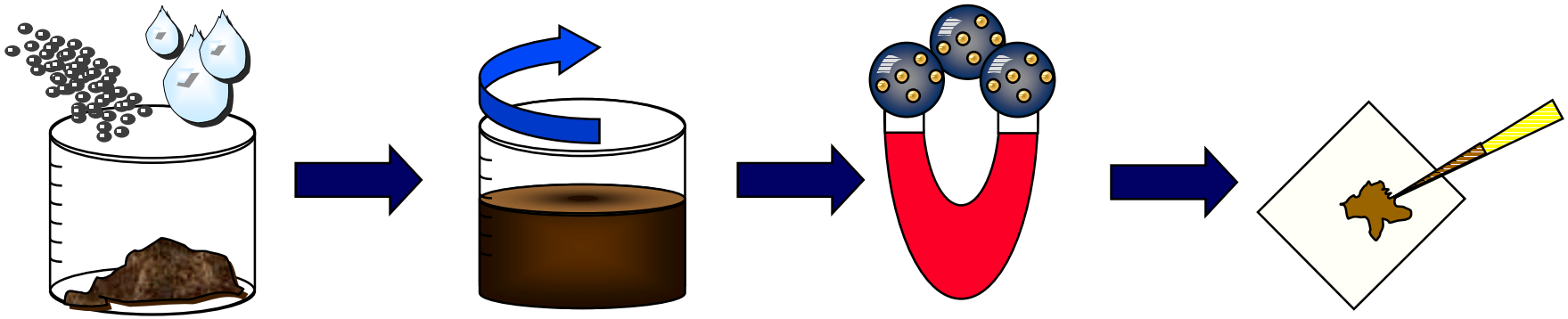


5) Signal Analysis and Readout

Target Concentration: Affinity Magnet Protocol

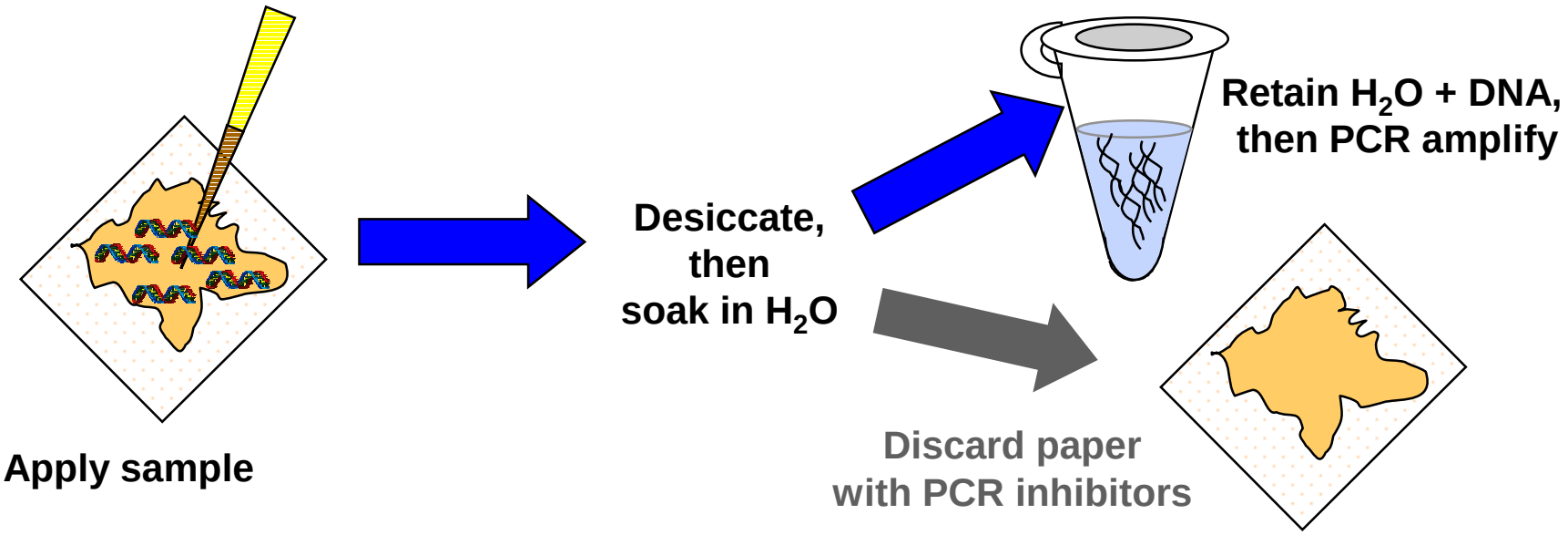






DNA Purification: Simple Nucleic Acid Prep (SNAP)

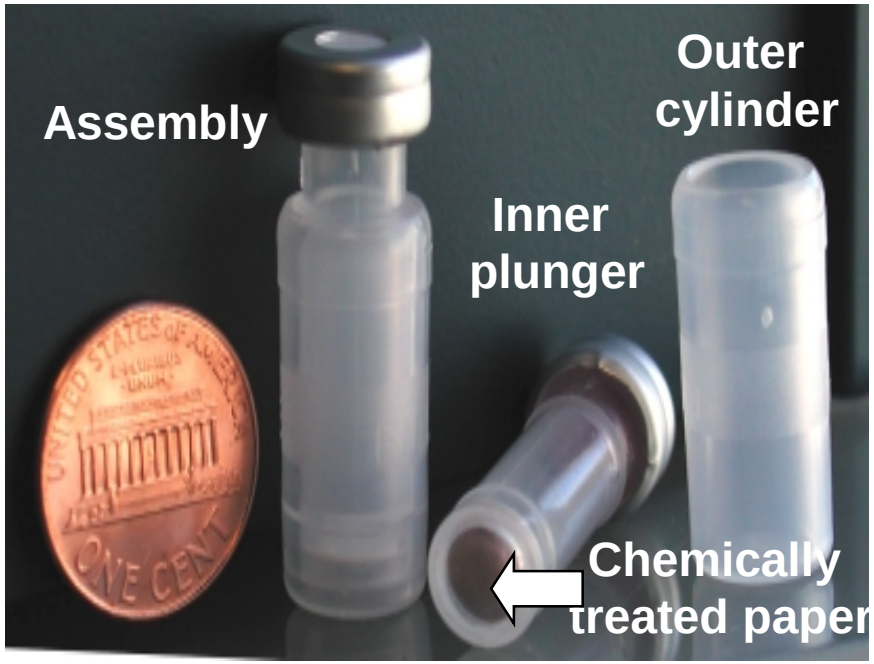
- Chemically treated paper is the key component of SNAP
- Lyses cells, binds PCR-assay inhibitors, and purifies DNA
- Advantages:
  - Fast and easy (1/5th the time of other published protocols)
  - Water is only added reagent (no phenol, chloroform, or alcohol)
  - Lightweight, compact, enables archiving
  - On-site fixation: preserves DNA & kills pathogenic organisms



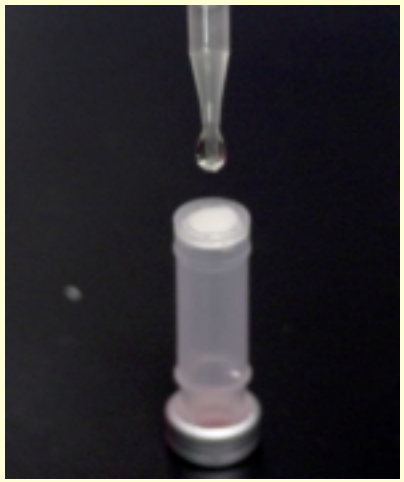
Lincoln Interim Nucleic-acid Kit (LINK)

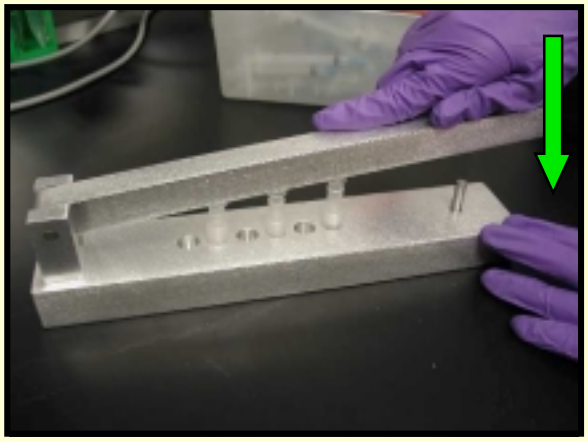
(Developed in response to October 2001 events)

- **LINK as a solution:**
  - **Incorporates SNAP paper but in a more user-friendly format**
  - **Faster processing than basic SNAP**
  - **Easier to sample, handle, and process**
  - **Enables on-site fixation**
  - **Outside can be decontaminated**
  - **6 minute processing time**
  - **Single-step processing**
  - **Results equal to or better than basic SNAP**



How to Use LINK





1) Apply sample
Sit for 5 minutes

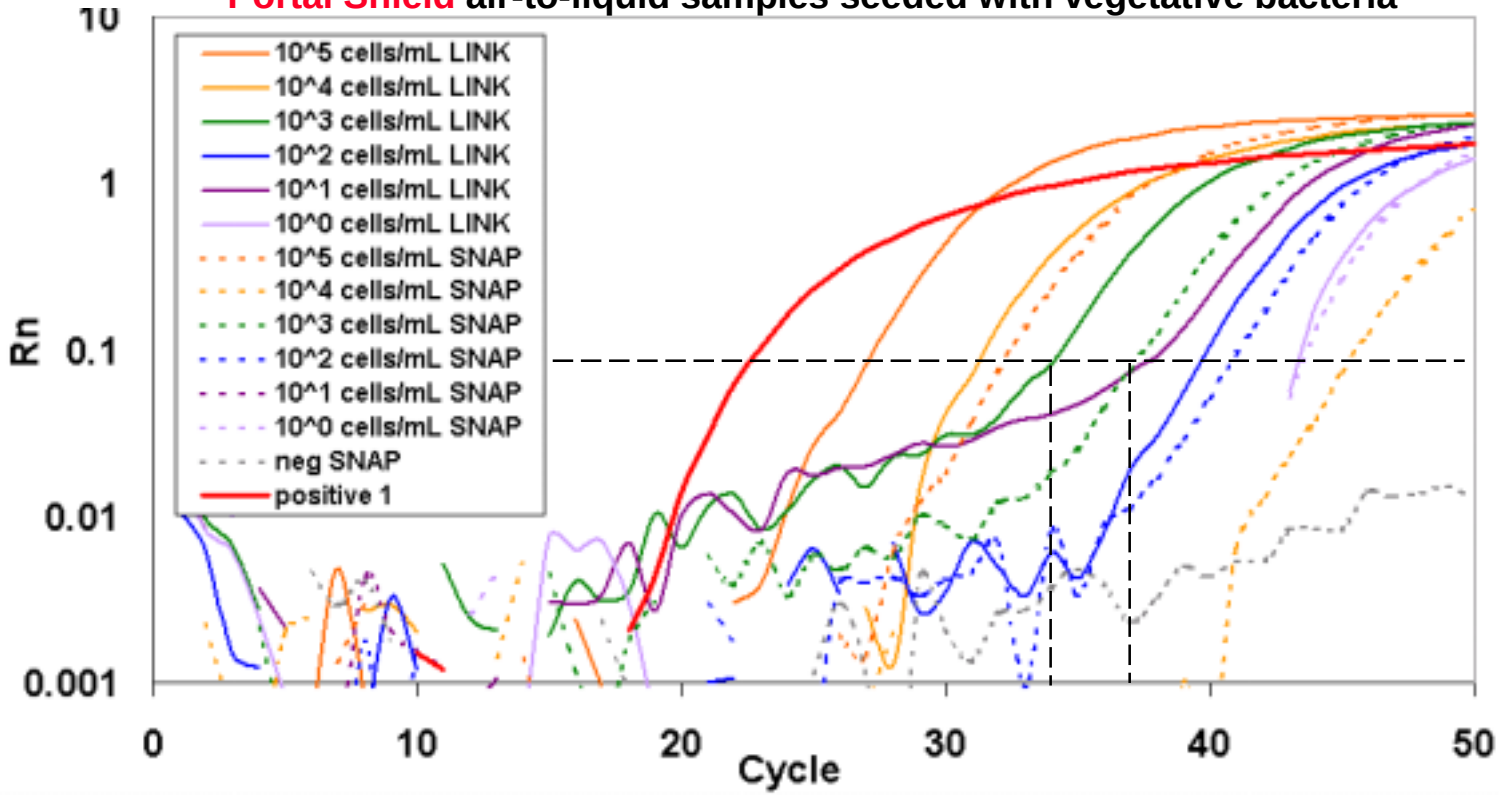
2) Process in one step

3) Remove DNA
Total time ~6 minutes!

LINK Cartridge Works with Varied Samples

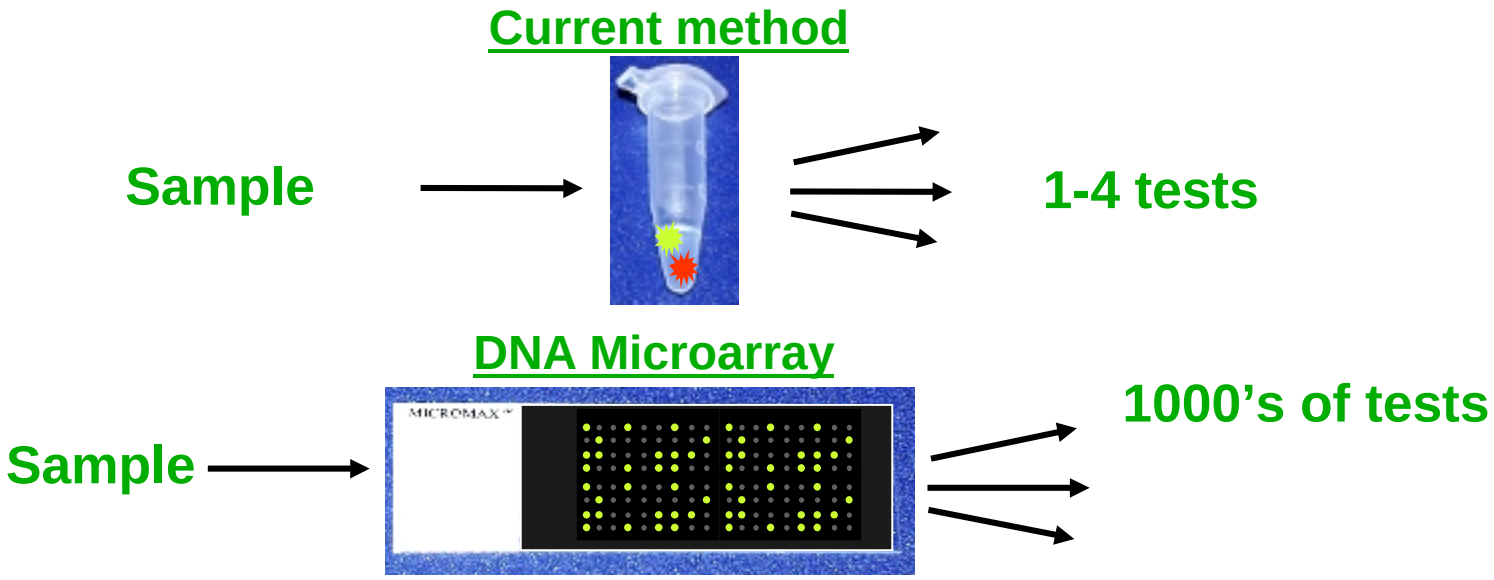
- **LINK detection from:**
  - **Portal Shield air-to-liquid samples seeded with vegetative bacteria**
  - **Untreated domestic sewage (Boston) seeded with vegetative bacteria**
  - **Paper, envelopes, skin seeded with bacterial spores**
  - **Air impaction with dry bacterial spores**

Portal Shield air-to-liquid samples seeded with vegetative bacteria



What About DNA Microarrays?

- DNA Microarray: Any 2D or 3D substrate having many (~ 10^2-10^5) different nucleic-acid capture sites (probes)
- Can identify both strain and drug resistance of pathogens
- Can offer highly multiplexed assay capability



Pathogen Identification via DNA Microarray

- **Detect small amounts (<100 copies per ml) of pathogen-specific nucleic acids in environmental sample**

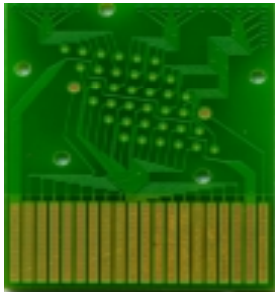
- **Arrays might provide log orders more information than current PCR-based approaches (e.g.TaqMan)**

- **Challenges for diagnostic applications:**
  - **Never demonstrated for environmental (or clinical) samples**
  - **Amplification may be necessary before micro-array assay**
  - **Sample preparation required (as in PCR techniques)**

Assay Times for Current and Emerging PCR/DNA Systems

< 1 Hour

- **Motorola**
- **Nanogen**
- **Host Genotype**



1 –2 Hours

- **Cepheid PCR**
- **Roche PCR**
- **Host Genotyping**
- **10's expressed RNAs**
- **10's pathogen genes**

2-4 Hours

MICROARRAYS

- **Expression Profiles**
- **Host Genotyping**
- **100's Pathogen genes (*)**

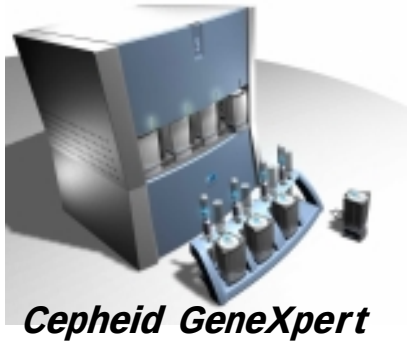
(*) w/ PCR

12+ Hours

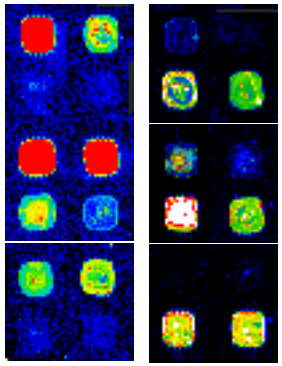
MICROARRAYS

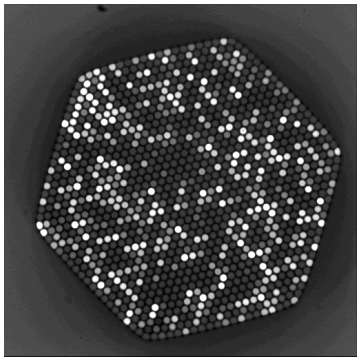
- **Expression Profiles**
- **Host Genotyping**
- **1000's Pathogen genes (#)**

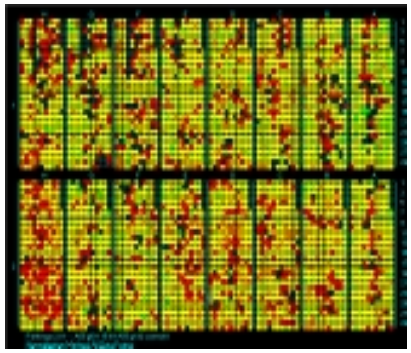
(#) w/culture



Cepheid GeneXpert







Summary

- **Environmental sample analysis parallels methodology developed for clinical sampling**
  - **Immunoassays for rapid estimate of exposure (not yet CDC authorized)**
  - **PCR techniques being deployed in some laboratories to provide strain specificity and drug resistance**
  - **Culture still used to provide "gold standard" for pathogen ID**
- **New technology developments could greatly increase the speed, sensitivity, and multiplicity of environmental assays**
  - **Protein microarrays could offer highly multiplexed, rapid ID capability on collected samples**
  - **DNA microarrays could offer hundreds to thousands of pathogen tests on single-chip format, provided sample preparation can be made compatible**